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(19) (CA) # CANADIAN PATENT (12)

(54) RAPESEED PROTEIN DETOXIFICATION AND RECOVERY

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This invention is for the extraction of protein and the elimination of goitrogenic substances normally present in protein extracted from rapeseed flour or meal. The process produces a product containing only about 6 µg/g 4-pentenyl isothiocyanate (99.95% toxin elimination) suitable for human consumption, as well as extracted meal which is useful as animal feed. The invention includes two major steps for detoxification:

1. Myrosinase treatment to hydrolyze glucosinolates to aglycones (isothiocyanates and goitrin);
2. Activated carbon adsorption of the aglycones.

Although activated carbon treatment for decolorization and deodorization is popular in the food industry, its utilization for aglycone removal as in this application is believed innovative, and the degree of detoxification achieved is unexpectedly high.

A number of methods for removal of potentially goitrogenic glucosinolates from de-oiled rapeseed meal have been proposed. Approaches taken to date include:

1. Removal of volatile substances by heating and steaming without prior breakdown of glucosinolates.
2. Direct chemical degradation and microbial destruction of glucosinolates.
3. Hydrolysis of glucosinolates and removal of products by distillation.
4. Deactivation of the myrosinase enzyme system.
5. Extraction of glucosinolates and hydrolysis products.

Compared to these prior approaches, our method is believed superior, since the efficiency of detoxification is extremely high with minimum denaturation effect on extracted proteins because of mildness of the treatment. The recovered proteins have nutritional and functional properties

equivalent to the best prior rapeseed protein products. This method should be reasonably economical.

Our process comprises the following steps:

(a) extracting the rapeseed flour or meal with an aqueous extractant preferably at alkaline pH of up to about 12, and separating the soluble material from the residual meal,

(b) treating the proteinaceous material (flour, meal or extract) in an aqueous medium preferably at a pH of about 7 to 9 with myrosinase to hydrolyze the glucosinolates present to aglycones,

(c) contacting a solution or dispersion of the protein plus aglycones, at neutral or extraction pH, (e.g. pH about 7 to 12) with activated carbon to adsorb the aglycones thereon, separating the protein from the carbon solids, and

(d) recovering substantially detoxified protein concentrate and residual meal.

Normally, oil is extracted first and the present process applied to the oil-extracted meal. However, this process is applicable to any ground rapeseed or proteinaceous fraction thereof.

The pH of the aqueous extractant may be any except the isoelectric precipitation zone (pH 2 to 7) with the yield of protein extracted varying with pH. An alkaline pH has been found most suitable. The greatest yields of protein are obtained at alkaline pH within the range of about 8.5 to 10.5 and this pH range is preferred. Protein fractions having slightly different properties may be obtained at other pH's if desired. At least one extraction will normally be carried out at alkaline pH. Protein extracted at pH 8.5 to 10.5 will precipitate at about pH 3.6 to 4.0 (isoelectric point) and a protein isolate can be prepared in this way. The protein isolate can be redissolved

or dispersed at the neutral or extraction pH's for detoxification or other processing.

Alternatively, the protein can be concentrated and/or isolated from solution by water removal, e.g. by one or more of evaporation, reverse osmosis, and ultrafiltration.

The myrosinase enzyme used to hydrolyze the thioglucosides or glucosinolates can be obtained from ground mustard seed e.g., by cold aqueous extraction optionally with an additional step of purification by ethanolic precipitation. Still other plant sources are possible. The amount of enzyme added is at least sufficient to effect the hydrolysis, usually about 0.1 to 1.5% as mustard seed used for enzyme extraction based on the original rapeseed meal used for protein extraction. The most suitable amount will depend on the variety of rapeseed, and the purity of the enzyme and its activity. The myrosinase is preferably utilized at a pH of about 7 to about 9, most preferably about 7.2. An acidic pH is operative but may lead to more toxic nitriles.

The adsorbent used to remove the toxic aglycones is any adsorptive carbon of high surface area which has been activated by techniques known to the art such as destructive distillation. Suitable adsorptive carbons or charcoals are known in the art. The minimum amount of carbon required will vary depending on its adsorptive capacity, the variety of rapeseed and whether a protein isolation step has been carried out. Usually, from about 8% to about 12% (based on the wt. of solids present) will be suitable for treating the extracts, and from about 3 to about 5% (based on the wt. of solids present) for treating the protein isolates. The used carbon can be

regenerated for re-use for example by heat treatment or solvent washing or both.

The adsorption of toxic materials on the carbon is more efficient at higher temperatures, i.e. is operative up to the boiling point. However, to minimize protein denaturation temperatures from ambient to 60°C will usually be used. The preferred temperature is about 45 to 60°C.

The yield of protein observed varied from about 50 to about 85%, e.g. 65% from rapeseed flour and 50% from industrial meal using isoelectric precipitation. A maximum yield of 83% was obtained using trichloroacetic acid for protein precipitation. The recovery of protein from the carbon detoxification step alone was high; no significant protein loss was observed.

One advantage of this rapeseed protein product is the absence of a "beany" flavour which is a drawback of soybean protein in some food applications. Flavour is especially improved by the carbon treatment resulting in a bland product surprisingly free of the typical rapeseed-type flavour.

The solution of detoxified protein, as separated from the carbon, can be spun, sheeted, spray-dried, texturized, or otherwise processed directly without prior recovery of dry protein.

Example

Rapeseed protein isolate from pH 10 NaOH extraction was analyzed by gas chromatography and UV absorption (C. G. Youngs and L. R. Wetter, 1967. J. Am. Oil Chem. Soc., 44: 551) and found to contain glucosinolates at levels equivalent to 0.66 mg 3-butenyl isothiocyanate, 0.51 mg 4-pentenyl isothiocyanate, and 0.4 mg oxazolidinethione (goitrin) per g isolate.

The two stage enzyme-carbon process was found to greatly decrease the levels of these toxins. Isolate slurry was treated at pH 7.2 with crude myrosinase extracted from white mustard seed to convert glucosinolates to isothiocyanates and goitrin, adjusted to pH 10, and the solution or dispersion contacted with granular activated carbon by a column method. Subsequent analysis revealed only 0.005 mg 4-pentenyl isothiocyanate per g isolate. Goitrin was not detectable. Infra-red analysis confirmed that the carbon was also effective in nitrile removal.

Preferred Steps and sequences with optional or alternative variations are now described.

A. Protein Extract Solution Preparation

1. Rapeseed flour or meal is ground preferably until it passes a 20 mesh screen.
2. Screened meal is slurried at neutral, acidic or basic pH for protein extraction.
3. Meal slurry is centrifuged to produce protein extract solution.

Extraction may be repeated to increase protein yield. The extracted meal may be used as cattle feed.

B. Preparation of Protein Isolate Slurry (Optional)

1. Protein extract (from A) is adjusted to isoelectric pH (which is a function of extraction pH) to precipitate protein.
2. Solution is centrifuged and protein isolate is collected.
3. Water is added to protein isolate to produce a protein isolate slurry.

4. Examples of Detoxification of Prepared Protein Extract (A) or Isolate (B)

Crude Enzyme and Carbon Column Method

a. Protein solution or slurry is adjusted to pH 7.2.

b. Crude myrosinase (thioglucoside (glucosinolate) glucohydrolase 3.2.3.1) prepared from white mustard seed (Sinapis alba) is added to the protein solution or slurry.

c. Mixture is stirred at pH about 7.2 (maintained with NaOH) for 3 hours.

d. Protein is resolubilized at extraction pH (if necessary).

e. Protein solution or dispersion is passed through a granular activated carbon column (equilibrated to pH of protein solution) preferably at 50°C to 60°C.

f. The protein solution is neutralized if necessary and the protein recovered.

Direct Carbon Addition

The process proceeds as before except that in step e, activated carbon (granular or powdered) is added to the protein solution. This mixture is stirred preferably at 50°C to 60°C for about 2 hours, then centrifuged or separated through a suitable screen to eliminate the carbon. Protein without solubilization can be similarly treated, where the carbon solids are readily separated from the protein solids as by screening etc.

Mustard Seed

As before except that in step b, ground white mustard seed (Sinapis alba), 1% by weight of

original rapeseed meal is added to the protein solution, as source of myrosinase. Also, in step e, the protein solution is centrifuged to remove spent mustard seed before carbon treatment.

Mustard Seed Addition Followed by Direct Carbon Addition

The process proceeds as before except that in step g, the final mixture is centrifuged to eliminate both carbon and mustard. Note: Second protein dissolution step may occur before or during activated carbon addition and 2 hour stirring period.

Crude Myrosinase and Direct Carbon Addition

As before except that in step b, activated carbon (powdered or granular) and crude myrosinase (as in 4b), are both added to the protein solution. Subsequently, in step e, the protein solution is centrifuged or screened to remove carbon.

Ground Mustard Seed and Direct Carbon Addition Together

As before except in step b, activated carbon (powdered or granular) and 1% (dry weight) ground white mustard seed is added. Subsequently, in step e, the protein solution is centrifuged or screened to remove both mustard and carbon.

Instead of the above sequence, direct addition of myrosinase or mustard seed to rapeseed meal is possible, with subsequent detoxification by column or batch procedure.

1 & 2. Screened ground meal is slurried with water and maintained at pH 7.2 with NaOH.

3. Ground white mustard seed (1%) or crude myrosinase is added to meal slurry.

4. The mixture is incubated as before to allow hydrolysis to proceed.

The resulting protein extract solution or isolate slurry is processed as before for detoxification and recovery of protein.

The carbon treatments can be carried out in 2 or more stages in column or batch (or both) fashion.

TEST RESULTS using the above procedures can be summarized as follows:

i. The isoelectric precipitation step and separation of mother liquor removed 89% of the glucosinolates.

ii. The carbon column detoxification step on the pH 10 soluble protein extract removed 95% of the aglycones.

iii. The carbon column detoxification step on the solution of protein isolate precipitated from the extract removed 99% of the aglycones (not including isoelectric precipitation step).

iv. The carbon column was found to be at least 93% effective (higher at intermediate and acidic pH) in aglycone removal in the range of pH 3 to 10 (without isoelectric precipitation).

v. Detoxified protein isolate (including isoelectric precipitation i) contained only 6 µg/g 4-pentenyl isothiocyanate signifying a detoxification efficiency of 99.96%.

A preferred procedure for overall economy is as follows:

Ground rapeseed meal (de-oiled) screened through 20 mesh screen is slurried at pH 10 for extraction. The protein extract after treatment to remove insolubles is adjusted to pH 4 and centrifuged to collect protein isolate. Protein isolate is slurried, adjusted to pH 7.2 and stirred with cold water extract of ground white mustard seed, 1% by weight of original rapeseed meal, for 3 hours. Then, granular (e.g. +40 mesh) activated carbon, at 3% of the solids weight in the slurry, is added and stirring continued for another 2 hours at 50°C. Non-protein insolubles are removed as by screening. Preferably, the filtrate after filtration through (e.g. 100 mesh) stainless steel screen is spray dried to recover protein solids.

Protein Feeding Test

Three groups of 10 weanling rats were fed for four weeks with casein as standard, and rapeseed protein isolates before and after detoxification according to the present invention. The isolates were prepared by extraction of de-oiled meal at pH 10, precipitation at pH 3.8 with hydrochloric acid, and part treated in solution with myrosinase at pH 7.2 followed by activated carbon treatment at pH 10, all at about 25°C. The protein efficiency ratios determined were as follows:

Table 1

	Casein	Untreated Rapeseed Protein	Detoxified Isolates
Protein Efficiency Ratio	2.50	2.32	2.38

An amino acid analysis of the same detoxified isolate gave the following values:

Table 2

	Rapeseed Protein Isolate (Detoxified with C)	Casein	Soybean Protein
	- g/100 g protein -		
Threonine	5.19	4.9	3.5
Valine	5.19	7.2	4.6
Cystine	2.55	0.3	1.5
Methionine	1.61	2.8	1.5
Isoleucine	4.06	6.1	4.6
Leucine	7.56	9.2	8.2
Phenylalanine	3.87	5.0	5.3
Tryptophan	1.30	1.7	1.4
Lysine	5.58	8.2	6.3

The essential amino acid index calculated according to Oser 1970 Jour. Amer. Oil Chem. Soc. 47: 453 which is a measure of the balance of essential amino acids for dietary purposes, was 74-77 for rapeseed protein, 75 for soybean protein and 91 for casein. Thus the biological efficiency for rapeseed protein is expected to be lower than casein and equivalent to or slightly better than that of soybean protein.

CLAIMS

1. A process for detoxification and recovery of rapeseed protein from rapeseed flour or de-oiled meal comprising:

(a) extracting the rapeseed flour or meal with an aqueous extractant for the protein, and separating the soluble material from the residual meal,

(b) hydrolyzing the glucosinolates present to aglycones in an aqueous medium with myrosinase,

(c) contacting a solution or dispersion containing the protein plus aglycones, at neutral or extraction pH, with activated carbon to adsorb the aglycones thereon, separating the protein from the carbon solids, and

(d) recovering substantially detoxified protein concentrate.

2. The process of claim 1 wherein the extraction (a) is at an alkaline pH of up to about 12.

3. The process of claim 1 wherein the enzyme hydrolysis (b) is at a pH of about 7 to 9.

4. The process of claim 1 wherein the protein is isolated from solution by precipitation at the isoelectric pH within about pH 2 to 7, in at least one step prior to step (c).

5. The process of claims 1, 2 and 3 wherein de-oiled rapeseed meal is extracted in step (a).

6. The process of claims 1, 2 and 3 wherein the enzyme hydrolysis (b) is at pH about 7.2.

7. The process of claim 2 wherein the alkaline pH is about 8.5 to 10.5.

8. The process of claims 1, 2 and 3 wherein the adsorption step (c) is carried out at about 45 to 60°C.

CLAIMS cont.

9. A detoxified rapeseed protein concentrate, soluble in aqueous media of alkaline pH, substantially free of oxazolidinethione, and containing not more than about 0.006 mg isothiocyanate per g, produced by the process of claims 1, 3 or 4 or their obvious chemical equivalents.

10. The detoxified concentrate of claim 9 having a protein efficiency ratio of approximately 2.4.

11. The detoxified concentrate of claims 9 and 10 having the amino acid content in g/100 g protein: threonine 5.19; valine 5.19; cystine 2.55; methionine 1.61; isoleucine 4.06; leucine 7.56; phenylalanine 3.87; tryptophan 1.30; and lysine 5.58.

TITLE

RAPESEED PROTEIN DETOXIFICATION AND RECOVERY

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ABSTRACT OF THE DISCLOSURE

Rapeseed flour or meal is extracted with aqueous alkaline liquid to solubilize protein, and this protein extract (or isolate therefrom) is treated with myrosinase preferably at pH about 7 - 9 to hydrolyze glucosinolates to aglycones. Alternatively, the myrosinase treatment may precede or accompany the extraction. At neutral or alkaline extraction pH, a solution or dispersion of the treated protein is contacted with activated carbon which adsorbs the aglycones. Detoxified protein of good nutritional and utilization properties is then recovered. The residual meal is useful as animal feed.